

# Increased rates of duodenal mucosal protein synthesis in vivo in patients with untreated coeliac disease

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## Abstract

**Background and Aims**—A robust, reproducible method for the measurement of protein synthesis in the gastrointestinal mucosa was applied to investigate possible differences between the rate of duodenal mucosal protein synthesis in coeliac patients and normal control subjects.

**Patients and Methods**—Eight patients, means (SD) (51 (10) years, 57 (11) kg, 160 (6) cm) with newly diagnosed untreated coeliac disease and seven control subjects (48 (11) years, 71.5 (12) kg, 172 (10) cm) received primed, continuous, intragastric (IG) and intravenous (IV) infusions of L-[1-<sup>13</sup>C]leucine and L-[1-<sup>13</sup>C]valine after an overnight fast. Distal duodenal biopsy specimens were obtained at endoscopy performed after 240 minutes of infusion. Protein synthesis was calculated from protein labelling relative to intracellular free amino acid enrichment, after appropriate mass spectrometric measurements. **Results**—Rates of duodenal protein synthesis were significantly greater in coeliac patients than in control subjects (IV tracer, coeliac *v* control, 3.58 (0.45) *v* 2.26 (0.22)%/h, *p*<0.05; IG tracer, 6.25 (0.97) *v* 2.34 (0.52)%/h respectively, *p*<0.01). The rates of mucosal protein synthesis calculated on the basis of the tracer infused via the intragastric route were higher in patients with coeliac disease than in control subjects. Tissue protein/DNA ratios were significantly reduced in coeliac patients (coeliac *v* control, 9.2 (1.6) mg/μg *v* 13.0 (2.2) mg/μg respectively, *p*<0.05) suggesting smaller mucosal cell size in coeliac patients.

**Conclusions**—Despite the villous atrophy and reduced cell size observed in coeliac disease, the rates of mucosal protein synthesis are considerably increased. These results suggest that a high rate of protein synthesis may be adaptive to a high rate of protein breakdown or mucosal cell loss in coeliac patients.

(Gut 1996; 39: 176-179)

Keywords: coeliac disease, protein synthesis, stable isotopes, mass spectrometry.

Coeliac disease (gluten sensitive enteropathy, non-tropical sprue) is characterised by: generalised malabsorption; a typical, but non-specific, small intestinal mucosal lesion; and a clinical and slower histological response to

withdrawal of gluten containing foods from the diet. The hallmark of coeliac disease is subtotal villous atrophy, abnormal epithelial cells on the mucosal surface, and increased cellularity of the lamina propria. Rates of epithelial cell renewal and migration in coeliac disease have been reported to be increased sixfold and the epithelial cells lining these crypts contain numerous mitotic figures.<sup>1 2</sup>

We have developed a safe, reproducible, and reliable method to measure protein synthesis in the gastrointestinal mucosa using branched chain amino acids labelled with <sup>13</sup>C<sup>3</sup> and used it to measure the rates of protein synthesis in the duodenal mucosa in patients with coeliac disease. No measurements of intestinal mucosal protein synthesis have previously been carried out in vivo in coeliac patients.

The main aims of this study were; (1) to measure the rate of small intestinal mucosal protein synthesis in untreated coeliac patients before the introduction of gluten free diet when the tracer was administered by intragastric (IG) and intravenous (IV) routes, (2) to compare the results with those results obtained from normal subjects, (3) to determine the effect of route of delivery on the rate of protein synthesis in coeliac patients.

## Methods

### Patients

Eight patients with newly diagnosed coeliac disease, four men and four women age 39-65 years, weight 40-67 kg (Table I) and seven control subjects (Table II) were studied. Each patient gave written consent after a full explanation of the study. Approval for the studies was obtained from the local ethics committee of Glasgow Royal Infirmary.

The criteria for diagnosis of coeliac disease were; clinical history of chronic diarrhoea with or without the passage of watery, pale stools with abdominal discomfort, distension, and weight loss; subtotal villous atrophy and inflammatory cellular infiltration of the lamina propria, mainly plasma cells and lymphocytes, in all patients as shown on jejunal biopsy specimens obtained by the Crosby-Kugler capsule<sup>4</sup>; clinical and histological response to the introduction of a gluten free diet. None of the subjects studied were taking any additional medication such as non-steroidal anti-inflammatory analgesics.

### Tracers

L-[1-<sup>13</sup>C]leucine and L-[1-<sup>13</sup>C]valine both 99

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Accepted for publication 27 February 1996

TABLE I Clinical data of normal subjects studied for duodenal mucosal protein synthesis

Patients	Age (years)	Sex	Weight (kg)	Height (cm)	% Ideal body weight
1	57	M	65	158	105.01
2	53	M	85	185	112.58
3	59	M	70	178	98.18
4	55	M	56	172	82.23
5	26	M	70	180	96.69
6	51	F	86	160	148.02
7	47	M	80	173	116.45

Atom % were obtained from MassTrace Incorporated, Woburn, MA, USA. Immediately before administration, the tracers were dissolved in sterile, non-pyrogenic 0.9% NaCl solution (150 mmol/l) (Baxter Health Care, Thetford, Norfolk, England) and were sterilised by passage through 0.20 micron filter (Acrodisc-DLL, Gelman Sciences, USA).

#### Experimental design

After an overnight fast, venous blood samples were taken, centrifuged and separated plasma was kept in liquid nitrogen. Priming doses of either L-[1-<sup>13</sup>C]leucine or L-[1-<sup>13</sup>C]valine (1 mg/kg body weight) were given IV over one minute; a constant infusion of the same tracers at a rate of 1 mg/kg body weight/h using 120 ml of 0.9% NaCl was then continued for four hours.

Simultaneously, the other tracer (either L-[1-<sup>13</sup>C]valine or L-[1-<sup>13</sup>C]leucine) was given via the enteral route. A priming dose (1 mg/kg body weight) in 50 ml 0.9% NaCl was delivered via a nasogastric tube (XRO-Paediatric Duodenal tube-39306, Vygon, Ecouen, France) over two minutes followed by a four hour constant infusion at 1 mg/kg body weight/h in 480 ml of 0.9% NaCl (120 ml/h), delivered via a pump (MHRE-7 Watson-Marlow Limited, Cornwall, England). The position of the nasogastric tube was checked radiologically and infusion only started after the tube was in a satisfactory position. After 240 minutes of continuous infusion, upper gastrointestinal endoscopy was performed and multiple distal duodenal biopsy specimens were obtained. The range of the pooled wet weight of specimens was 58.0-65.1 mg. Further biopsy samples were taken for histology and disaccharidase assay. Venous blood samples were taken at -15, 0, 60, 120, 180, and 240 minutes respectively and separated plasma kept in liquid nitrogen with the specimens.

#### Analysis

The labelling of plasma leucine, valine,  $\alpha$ -ketoisopropionate (KIC), and  $\alpha$ -ketoisovalerate

(KIV) were measured by standard gas chromatography mass spectrometry techniques using *t*-butyldimethylsilylation derivatives.<sup>5</sup> Mucosal tissue samples were frozen in liquid nitrogen immediately on sampling, and samples pooled for storage at -70°C. The labelling with <sup>13</sup>C of leucine and valine in hydrolysed protein was done by using preparative gas chromatography and isotope ratio mass spectrometry of the CO<sub>2</sub> liberated by ninhydrin.<sup>6</sup> Plasma protein from the pre-infusion samples was used to estimate basal body protein <sup>13</sup>C labelling for use in the calculation of mucosal protein synthetic rate.

The pooled mucosal tissue samples were analysed for concentration of protein, RNA, and DNA.<sup>7</sup>

The methods used have been validated in terms of linearity.<sup>3</sup>

#### Calculations and statistical analysis

Protein synthesis was calculated as fractional rate using the equation  $k_s$  (%h<sup>-1</sup>) =  $(E_t - E_0/E_p) \times 1/t \times 100$  where  $E_t$  is the enrichment in tissue protein at time  $t$ ,  $E_0$  is the baseline enrichment, and  $E_p$  is the enrichment of the precursor. The precursor we used in our calculation was the intracellular tracer amino acid. The calculation by this equation depends on the fact that the tracer incorporation into the protein is linear, which we have previously demonstrated.<sup>3</sup>

Whole body protein breakdown was calculated using the equation; Protein breakdown  $\mu$ mol of leucine/kg/h =  $i$  ( $\mu$ mol/kg/h)/ $E_{KIC}$ , where  $i$  is the rate of leucine infusion and  $E_{KIC}$  is the enrichment of the ketoacid.<sup>8</sup>

Values were expressed as means (SD). Statistical analysis used Wilcoxon's ranking test for the non-parametric, unpaired data (Mann-Whitney test). Differences were considered significant at  $p$  values of <0.05.

#### Results

A plateau level of 5 (0.27) Atom % excess of tracer enrichments was achieved within one hour of infusion for both IV and IG routes and remained so throughout the infusion period until biopsy specimens were obtained at endoscopy (data not shown).

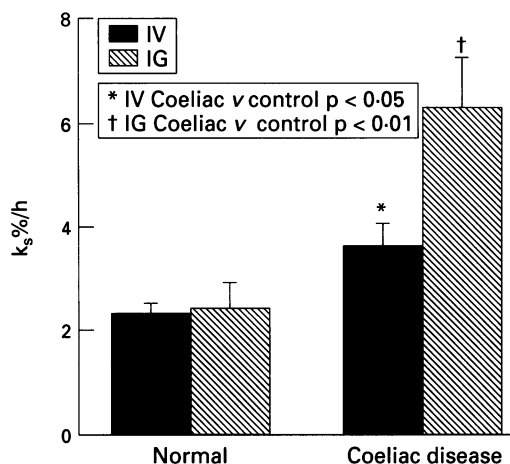
Distal duodenal mucosal protein synthesis rate was considerably increased in coeliac patients compared with control subjects whether determined by incorporation of the IV or IG infused tracers. IV tracer, coeliac *v* control 3.58 (0.45) *v* 2.26 (0.22) %/h,  $p < 0.05$ ; IG tracer 6.25 (0.52) *v* 2.34 (0.52) %/h,  $p < 0.01$  (Fig 1). Labelling of mucosal intracellular amino acids was higher when tracer was given IG than IV but there were no differences between the control subjects and coeliac patients, suggesting that the higher rate of protein synthesis measured with IG tracer in the coeliac patients was not the result of differential precursor labelling or luminal tracer malabsorption.

Protein/DNA ratio is reduced in the patients with coeliac disease compared with the control

TABLE II Clinical data of coeliac patients studied for duodenal mucosal protein synthesis

Patients	Age (years)	Sex	Weight (kg)	Height (cm)	% Ideal body weight
1	45	F	40	151	74.63
2	65	F	64	160	110.15
3	52	F	52	158	91.23
4	52	M	45	158	71.70
5	39	M	64	167	97.71
6	65	F	67	159	116.52
7	51	M	67	169	100.60
8	40	M	80	159	104.50

Figure 1: Small intestinal mucosal protein synthetic rate in patients with coeliac disease and control subjects expressed as  $k_s$  %/h after intravenous and intragastric delivery of tracer. These results were based on the enrichments of free amino acid tracer in the intracellular compartment.



subjects, 9.2 (1.6) mg/ $\mu$ g v 13.0 (2.2) mg/ $\mu$ g respectively,  $p < 0.05$ . The protein synthetic capacity, RNA/protein ratio, is increased in the coeliac patients compared with the control subjects, 172 (7.5)  $\mu$ g/mg v 137.1 (5.7)  $\mu$ g/mg respectively,  $p < 0.005$  (Fig 2).

Whole body protein breakdown in coeliac disease was 2.06 (0.16)  $\mu$ mol leucine/kg/h and in the control subjects 1.96 (0.44)  $\mu$ mol leucine/kg/h, the difference between the two groups not being statistically significant, suggesting that the effect of coeliac disease is localised to the intestinal mucosa.

### Discussion

This study presents for the first time the effect of untreated coeliac disease on the rate of small bowel mucosal protein synthesis in vivo. The rate of protein synthesis by the small intestinal mucosal cells was higher in coeliac patients despite the smaller size of the enterocytes. The increased rate of mucosal protein synthesis in coeliac patients is in agreement with the results of an in vitro study by Jones *et al.*<sup>9</sup> They cultured jejunal mucosa from patients with coeliac disease, treated coeliac disease and controls with <sup>14</sup>C-labelled leucine for 24 hours, and found that protein synthesis by mucosal cells from untreated coeliac patients was significantly greater than by control mucosa.

We calculated the mucosal protein synthesis as fractional rate relative to the intracellular tracer enrichment. Ideally the precursor should be the aminoacyl tRNA, which is difficult to measure.<sup>10 11</sup> Therefore, we used free mucosal intracellular tracer enrichment as a surrogate for tRNA. Presentation of the tracer amino acid to the apical (luminal) side of the enterocytes seems to be associated with higher protein synthetic rate than on presentation of these tracers on the basolateral (intravascular) side. This finding was unexpected and is not seen in normal healthy subjects, probably because of the preferential channelling of amino acids from the luminal side into the protein synthetic machinery, for reasons we do not understand. Nevertheless, the results suggest a high rate of protein synthesis from mucosal protein whichever tracer route is used.

Coeliac disease is caused by damage to the villous epithelium of the small intestinal mucosa, giving a characteristic histological

appearance<sup>12</sup> in response to the ingestion of dietary gluten, which is present in most cereal grains.<sup>13</sup> The condition may be accompanied by malabsorption of many nutrients, but the clinical picture only appears when the small intestinal damage is severe or deficiencies appear.<sup>14</sup> There is evidence of increased mucosal cell turnover with pronounced proliferation of normal crypt cells,<sup>15</sup> and our findings are in agreement with this. So far the metabolic capacity of these cells remains unclear.

Our diagnostic criteria for the diagnosis of coeliac disease depended on jejunal biopsy rather than distal duodenal biopsy. The patients improved clinically and histologically after the withdrawal of gluten from their diet, thus confirming the diagnosis.

The KIC/Leu and KIV/Val ratios were below 1 and were significantly lower in coeliac patients suggesting more dilution of the keto acids resulting from an increased rate of protein breakdown and turnover. The intestinal mucosa showed evidence of atrophy as manifested by the decreased protein/DNA ratio. The extrapolation that the protein/DNA ratio reflects epithelial cell size has been confirmed by conducting histomorphometric studies on the small intestinal mucosal biopsy specimens from the coeliac patients and the control subjects (unpublished data). The capacity of protein synthesis (RNA/protein) is increased in the coeliac patients. This is probably a reflection of the immaturity of the enterocytes lining the villus or increased rate of mucosal protein breakdown, or both. In conclusion the small intestinal mucosal cells in coeliac disease seem to be immature, hence there is poor development of absorptive and metabolic functions.

The results of this study provide further insight into the changes in protein metabolism at tissue level that occur in patients with coeliac disease. The tissue biopsy specimens in these studies are made up, not only of intestinal

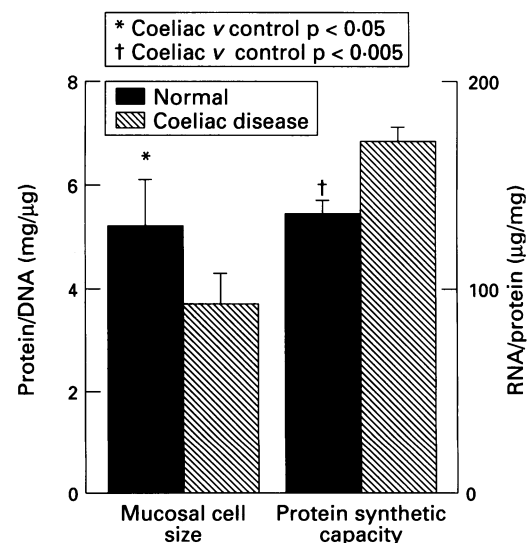


Figure 2: Small intestinal mucosal cell size expressed as protein/DNA ratio in patients with coeliac disease and normal subjects. The capacity of these mucosal cells to synthesise protein is expressed as RNA/protein ratio. In coeliac patients the mucosal cells were of smaller size but of higher capacity for protein synthesis.

epithelial cells but also comprise many other cell types, for example, mesenchymal cells, immunocytes, and fibrous tissue. The contribution of the lamina propria to the protein synthesis is negligible, as the rate of mucosal protein synthesis was the same in all the untreated coeliac patients despite the varying degree of inflammatory cellular infiltration in those patients. Furthermore, it has been found by analytical subcellular fractionation that most of the net protein synthesis takes place in the enterocytes and only negligible amounts synthesised by the cells of the lamina propria.<sup>16</sup> Further developments of the current technique of measuring mucosal protein synthesis *in vivo* is required to allow the contribution of each cell type to the rate of protein synthesis in the whole biopsy specimen to be determined.

Jones *et al* found that protein synthesis by treated non-responsive coeliac mucosa was significantly less than untreated coeliac mucosa but greater than control mucosa.<sup>9</sup> Further *in vivo* studies are required to re-evaluate the rate of protein synthesis in patients with coeliac disease who have responded clinically and histologically to a gluten free diet as well as in those patients who have not responded to this diet.

This work was supported by The Wellcome Trust, the University of Dundee, and the Medical Research Council.

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