

# Glycoprotein synthesis and secretion by cultured small intestinal mucosa in coeliac disease

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**SUMMARY** Glycoprotein biosynthesis by jejunal mucosa was examined during culture *in vitro* in 26 patients with coeliac disease and 19 controls. The incorporation rates of tritiated glucosamine into tissue and secreted glycoproteins were determined using established techniques. The total glucosamine incorporation in untreated coeliac patients was significantly greater than that of histologically normal mucosa ( $p < 0.001$ ) and jejunal tissue from patients with treated coeliac disease ( $p < 0.01$ ). Enhanced secretion of *in vitro* labelled glycoproteins was observed in untreated coeliac patients. The total incorporation of tritiated glucosamine in intestinal tissues was correlated with goblet cell numbers. These results indicate that quantitative changes in glycoprotein synthesis and secretion occur in coeliac disease.

In coeliac disease many immunological, biochemical, and morphological changes are evident in the jejunal mucosa of patients with villous atrophy. Specific abnormalities associated with the epithelium include changes in enterocyte structure,<sup>1</sup> brush border enzyme activity,<sup>2</sup> crypt cell production rate<sup>3</sup> and HLA antigen expression.<sup>4</sup> Little, however, is known about goblet cell function in coeliac disease and, surprisingly, there have been no biochemical or functional investigations of mucus glycoprotein secretion in patients with coeliac disease. The mucus barrier is an important factor in the protection of the intestinal epithelium from luminal aggressors<sup>5</sup> and qualitative and quantitative changes in mucus glycoprotein synthesis and secretion have been associated with specific intestinal diseases.<sup>6-8</sup>

Immunological factors have been shown to stimulate mucus glycoprotein secretion at pulmonary<sup>9,10</sup> and gastrointestinal sites.<sup>11</sup> There is strong evidence from immunohistological and functional studies of raised immunological activity in the small intestinal mucosa of untreated coeliac patients.<sup>12,13</sup> Enhanced mucosal immunological activity could well influence small intestinal glycoprotein biosynthesis. In this study we have investigated glycoprotein synthesis and secretion by small

intestinal mucosa of patients with coeliac disease and normal subjects during short term *in vitro* culture.

## Methods

### COELIAC PATIENTS

Jejunal biopsies were obtained from 26 patients with coeliac disease using a Crosby capsule or a Quinton hydraulic multiple biopsy instrument. Thirteen were untreated (10 women, mean age 39.1 years, range 17-69) on normal diets at the time of biopsy. Jejunal biopsies from these patients showed subtotal villous atrophy on histological examination. Ten of the untreated patients have, to date, shown histological improvement of villous architecture on gluten free diets. The 13 treated coeliac patients (11 women, mean age 36.9 years, range 17-53) who were studied, had been on gluten free diets for a minimum of six months at the time of biopsy. Biopsies from eight patients were histologically normal and five patients had partial villous atrophy.

### CONTROL PATIENTS

Jejunal tissue was obtained from 19 control patients (mean age 34.6 years, range 17-60) undergoing investigation for possible coeliac disease. Multiple biopsies were also obtained from the second part of the duodenum of 39 patients at endoscopy. The duodenal and jejunal mucosae of all control patients were normal at histological examination.

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## ORGAN CULTURE

Immediately after biopsy, jejunal tissue was divided into pieces for routine histology and *in vitro* culture, using aseptic techniques. Biopsies for culture were placed into culture medium consisting of RPMI 1640 containing 23.8 mM sodium bicarbonate (Flow Laboratories Ltd., Rickmansworth, Herts.), 40 µg/ml gentamicin and 10% heat-inactivated fetal calf serum (Sera-Lab Ltd., Crawley). Each biopsy (two per patient) was orientated mucosal surface upwards on sterile stainless steel grid positioned over the central well of an organ culture dish (Falcon) containing 1.6 ml of culture medium. The biopsies were incubated in a 5% CO<sub>2</sub> humidified incubator at 37°C. Duodenal biopsies were treated similarly.

## GLYCOPROTEIN SYNTHESIS AND SECRETION

The incorporation of radiolabelled glucosamine into acid-precipitable glycoproteins was measured using modifications of the methods of MacDermott *et al.*<sup>14</sup> Biopsies were cultured in duplicate in medium containing 5 µCi of D-[1-<sup>3</sup>H]-glucosamine hydrochloride (specific activity 5.8 Ci mmol; Amersham). At the end of culture, the biopsies were gently rinsed in medium devoid of radiolabel to remove surface mucus. The tissue was homogenised in 2 ml 3.3 mM CaCl<sub>2</sub> at 4°C. Two aliquots (200 µl) of the homogenate were removed for determination of total protein by a modified Lowry method<sup>15</sup> and the glycoproteins in a further aliquot (1400 µl) were precipitated with 10% trichloroacetic acid (TCA) and 1% phosphotungstic acid (PTA) at 4°C overnight. After centrifugation (10000 g) the precipitate was washed three times with 10% TCA and 1% PTA to remove unincorporated glucosamine and twice with 1:1 chloroform-methanol to extract lipids. The dried acid-insoluble precipitate was solubilised in 1 ml NCS (Amersham) and counted in liquid scintillation solution (Optiphase X) in a Packard Tricarb 4000 liquid scintillation counter. To correct for quenching, the samples were counted against an internal standard and the results expressed as disintegrations per minute per mg biopsy protein (dpm/mg protein).

The secreted radiolabelled glycoproteins were measured by precipitating with 10% TCA and 1% PTA the glycoproteins in the culture medium and the medium used to rinse the biopsy. The precipitates were processed and counted in a similar manner to tissue glycoproteins TCA-PTA precipitates. Control cultures of radiolabelled medium incubated with no biopsy tissue were similarly processed to correct for non-specific binding of radioactivity to fetal calf serum. Total incorporation of radiolabel into acid precipitable glycoproteins was determined by combining the tissue and secreted counts. Data are

expressed as means standard errors (SE) of duplicate cultures. Analysis of variance was carried out using the Kruskal-Wallis test. Statistical comparisons between mean values were carried out using the Mann-Whitney U-test for non-parametric data.

## MORPHOLOGICAL STUDIES

To examine the relationship between glycoprotein synthesis and secretion and goblet cell numbers in individual patients, morphometric analysis of histological sections of jejunal tissue adjacent to that used for culture was undertaken. Periodic acid-Schiff stained sections were projected onto graph paper using a Leitz projecting microscope from a fixed distance. The area of the longitudinally sectioned biopsies from the epithelium to the submucosa was outlined and the number of goblet cells recorded. The area of the section was determined by comparison of the weight of the outlined area on the graph paper to that of a standard area determined by a graticule calibration slide. Triplicate or quadruplicate counts of non-overlapping areas were made of appropriately longitudinally sectioned biopsies and the results expressed as goblet cells/10<sup>6</sup> µm<sup>2</sup> of mucosa. Goblet cell numbers were also expressed per unit length (10 mm) of epithelium.

## Results

The incorporation of <sup>3</sup>H glucosamine into glycoproteins by histologically normal duodenal tissue during 24 and 48 hours culture *in vitro* is shown in the Table. The total incorporation into tissue and secreted glycoproteins appeared to be linear over a 48 hour culture period. The counts associated with the tissue fraction increased between 24 and 48 hours culture suggesting continued glycoprotein synthesis. The percentage of radiolabelled glycoproteins secreted into the culture medium increased with the period of culture, 27% and 42.9% of the radiolabel being associated with secreted glycoproteins at 24 and 48 hours respectively.

Table Incorporation of <sup>3</sup>H glucosamine into tissue and secreted glycoproteins by duodenal biopsies during culture *in vitro*

Culture (h)	<sup>3</sup> H glucosamine incorporation DPM/mg protein × 10 <sup>3</sup> (Mean (SE))			
	n	Tissue	Secreted	Total incorporation
24	27	144 (7.4)	53.5 (4.8)	197.5 (8.7)
48	12	225 (36.7)	175.5 (30)	400.5 (61.8)

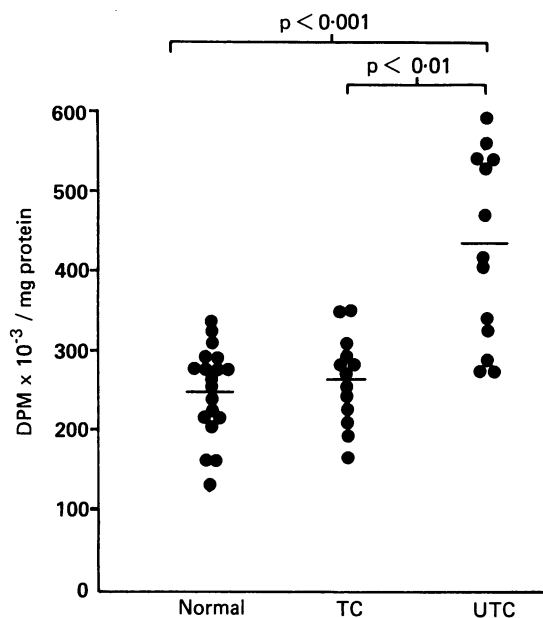


Fig. 1 Total incorporation of  $^3\text{H}$  glucosamine into acid precipitable glycoproteins by normal jejunal mucosa and mucosa from treated (TC) and untreated (UTC) coeliac patients during 24 hours culture *in vitro*.

The total incorporation of exogenous  $^3\text{H}$  glucosamine into tissue and secreted glycoproteins by coeliac and normal jejunal tissue over 24 hours culture *in vitro* is shown in Figure 1. The total incorporation by untreated coeliac mucosa, 427 (34) dpm  $\times 10^3$ /mg protein (mean (SE)), was significantly greater ( $p < 0.001$ ) than that of histologically normal mucosa, 248 (13) dpm  $\times 10^3$ /mg protein. Glycoprotein synthesis and secretion by treated coeliac mucosa, 265 (15.3) dpm  $\times 10^3$ /mg protein, was not significantly different from that of normal jejunal tissue, but was significantly lower ( $p < 0.01$ ) than that of untreated coeliac mucosa.

The proportion of  $^3\text{H}$  glucosamine incorporated into tissue and secreted glycoproteins is shown in Figure 2. Biopsies from untreated coeliac patients incorporated significantly higher amounts ( $p < 0.05$ ) of radiolabel into tissue glycoproteins than normal or treated coeliac mucosa. The secretion of radiolabelled glycoproteins by untreated coeliac mucosa was also significantly greater than normal ( $p < 0.001$ ) and treated coeliac ( $p < 0.01$ ) tissue. In untreated coeliac mucosa, 39.5% of the  $^3\text{H}$  glucosamine incorporated into acid precipitable glycoproteins over a 24 hour period was secreted. The percentage of radiolabelled glycoproteins secreted by normal and treated coeliac tissue was significantly lower

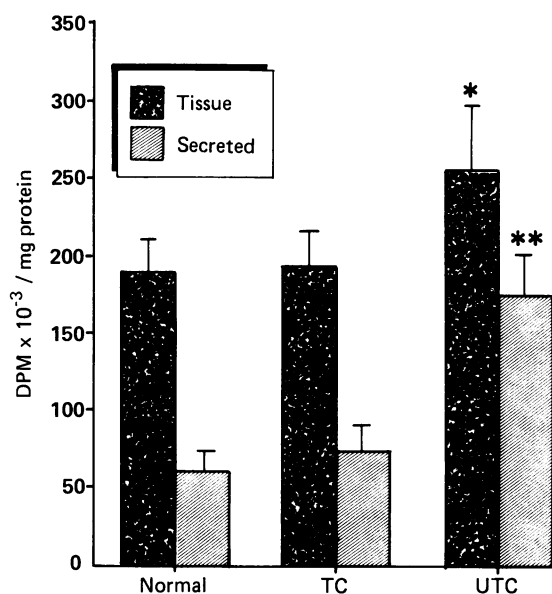


Fig. 2 Incorporation of  $^3\text{H}$  glucosamine into tissue and secreted glycoproteins by normal jejunal mucosa and mucosa from treated (TC) and untreated (UTC) coeliac patients during 24 hours *in vitro* culture. \* $p < 0.05$  from controls \*\* $p < 0.01$  from TC and  $< 0.001$  from controls.

( $p < 0.05$ ), being 24% and 27% respectively.

There was a significant correlation ( $p < 0.05$ ) between the total  $^3\text{H}$  glucosamine incorporation into glycoproteins in histologically normal jejunal tissue and the goblet cell numbers per unit area of mucosa in histological sections of adjacent tissue (Fig. 3). The mean goblet cell numbers/ $10^6 \mu\text{m}^2$  in histologically normal mucosa were not significantly different from those in untreated coeliac mucosa being 358 (22.3) and 388 (24) respectively. When goblet cell numbers were expressed per unit length of epithelium (10 mm), the mean cell number in untreated coeliac mucosa (299.5 (13.9)) was significantly higher ( $p < 0.01$ ) than that of histologically normal mucosa (223.5 (12.9)).

## Discussion

The results show that over 24 hours culture *in vitro*, untreated coeliac mucosa synthesises and secretes greater quantities of glycoproteins than histologically normal jejunal mucosa. The use of radiolabelled sugars to measure gastrointestinal glycoprotein production is a well established technique<sup>7,14,16</sup> and is thought largely to be an indication of mucus glycoprotein secretion.<sup>14</sup> Small intestinal brush border membrane proteins have, however, been shown to

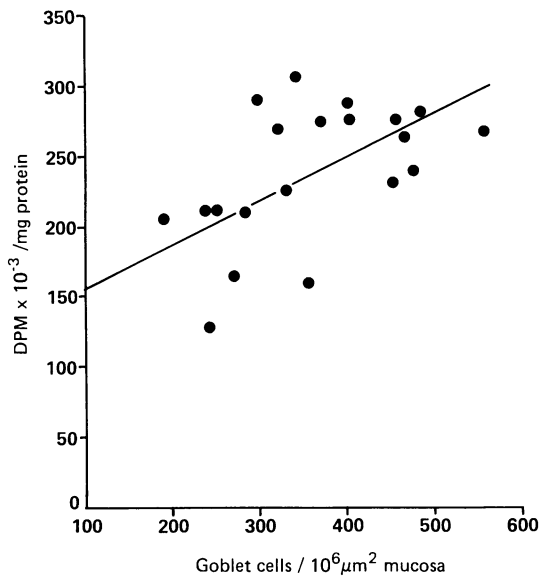


Fig. 3 Relationship between total incorporation of  $^3\text{H}$  glucosamine into glycoproteins during 24 hours culture and goblet cell numbers per unit area in histologically normal jejunal tissue.  $p < 0.05$ ,  $r = 0.533$ .

incorporate radiolabelled glucosamine during culture.<sup>17</sup> In the latter study the majority of radiolabelled glycoproteins were in a high molecular weight fraction with no brush border enzyme activity.<sup>17</sup> The reduction in intestinal brush border enzyme activities in untreated coeliac patients<sup>18</sup> implies that the enhanced glycoprotein synthesis observed in this group is not attributable to labelling of such components, although it could be indicative of increased cell turnover.<sup>3</sup> Furthermore, evidence from animal studies suggests that differentiated upper villous enterocytes incorporate more labelled sugar precursors into membrane glycoproteins than undifferentiated crypt cells.<sup>19</sup> Previous *in vitro* studies with  $^{14}\text{C}$ -labelled leucine have demonstrated enhanced protein synthesis in untreated coeliac mucosa<sup>20</sup> and the incorporation of radiolabel during 24 hours culture was linear for both normal and coeliac mucosa suggesting no differential survival of tissue during culture *in vitro*.

The results of this study show a significant correlation between  $^3\text{H}$  glucosamine incorporation into glycoproteins in histologically normal tissue and goblet cell numbers/ $10^6 \mu\text{m}^2$  mucosa in individual patients. Whilst the number of goblet cells per unit length of epithelium was significantly increased in untreated coeliac mucosa, the changes in surface to mucosal area ratio associated with villous atrophy resulted in no marked increases in goblet cell number

when expressed per unit area of mucosa. These observations suggest that the increased glucosamine incorporation in untreated mucosa (expressed as dpm/mg biopsy protein) could in part be a result of an increase in the rate of mucus glycoprotein biosynthesis at the cellular level. While local immunoglobulin production is increased in coeliac disease,<sup>13, 21</sup> the extent of glycosylation of immunoglobulins<sup>22</sup> is unlikely to account for the markedly increased incorporation of  $^3\text{H}$  glucosamine into glycoproteins over the 24 hour culture period. Alternatively, the differences in incorporation could reflect changes in precursor pool sizes, with histologically normal mucosa having a larger intracellular pool of unlabelled glucosamine. Free glucosamine, however, had not been found in intestinal tissue.<sup>23</sup>

Several immunological factors could contribute to the enhanced secretion of radiolabelled glycoproteins observed in untreated coeliac patients. Immune complexes,<sup>11</sup> macrophage products<sup>9</sup> and the anaphylatoxin C3a<sup>24</sup> have all been shown to stimulate mucus glycoprotein secretion by intestinal or pulmonary tissue. T lymphocytes have also been implicated in intestinal goblet cell hyperplasia in helminth infections in rats.<sup>25, 26</sup> The increased activation of T lymphocytes in untreated coeliac mucosa<sup>12, 13</sup> may induce changes in goblet cell maturation or differentiation. T lymphocyte products have recently been shown to modify epithelial differentiation, enhancing the expression of HLA-DR on intestinal epithelial cell lines<sup>27</sup> and fetal intestinal epithelium<sup>28</sup> *in vitro*.

The basal rate of mucus secretion can also be modified by endogenous neurotransmitters – for example, acetylcholine,<sup>29</sup> and vasoactive amines such as 5-hydroxytryptamine<sup>30</sup> as well as numerous external secretagogues.

Whilst our results show quantitative changes in mucus glycoprotein secretion in untreated coeliac mucosa, relatively little is known about the biochemical nature of mucus in coeliac disease. Certainly in untreated patients there is an increase in intestinal permeability,<sup>31</sup> suggesting that the enhanced mucus secretion does not impede the uptake of marker molecules. A recent histological study has reported changes in lectin binding characteristics of goblet cells of children with coeliac disease, implying alterations in oligosaccharide side chains of mucus glycoproteins.<sup>32</sup> In situations in which increased mucus glycoprotein secretion is observed, qualitative changes in the released mucus frequently occur. This, therefore, may have direct relevance to the pathogenesis of coeliac disease, accounting for the observed increase in intestinal permeability and perhaps also some of the recognised immune abnormalities in this condition.

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