# Electrogenic glucose absorption in untreated and treated coeliac disease

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SUMMARY    Using a method for measuring changes in transmural potential difference across the human jejunum *in vivo*, the operational kinetic parameters of 'Apparent Km' and PD max for the active electrogenic component of glucose absorption were estimated in a group of healthy volunteers and in patients with coeliac disease. Both the 'Apparent Km' ($17 \pm 2$ mM; mean $\pm$ SEM) and the PD max ($8.6 \pm 0.7$ mV) in nine patients with untreated coeliac disease were significantly lower ($p < 0.005$) than in the control group ('Apparent Km' $= 74 \pm 5$ mM; PD max $12.8 \pm 0.9$ mV, n $= 20$). Treatment of five coeliac patients by gluten withdrawal for less than three months increased significantly the values of both the 'Apparent Km' ($35 \pm 6$ mM) and the PD max ($11.4 \pm 1.2$ mV). Treatment of five patients for more than six months caused a further increase in the values of both kinetic parameters ('Apparent Km' $= 108 \pm 13$ mM; PD max $= 15.6 \pm 2.7$ mV) to levels which exceeded those in healthy subjects. The possible interpretations of the differences in the kinetic characteristics of electrogenic glucose transport between coeliac patients and healthy subjects are discussed.

In a previous study we described a technique for characterising the active electrogenic component of glucose absorption in man (Read *et al.*, 1974). We have now applied this method to investigate changes in electrogenic glucose absorption in patients with active and treated coeliac disease.

## Methods

### SUBJECTS

Tests of electrogenic glucose absorption were carried out on a group of 10 male and 10 female young healthy volunteers and on patients with coeliac disease, who were diagnosed on the criteria of clinical evidence of malabsorption, the typical biopsy appearance of total or subtotal villous atrophy, and response to treatment by gluten withdrawal. In the coeliac group tests were carried out on nine patients who had received no treatment, five patients treated for less than three months, and five patients treated for more than six months. These subgroups included the patients in Table 3 who were tested on more than one occasion. All the treated patients had evidence of either clinical or histological

response to withdrawal of gluten at the time of testing.

The method for characterising electrogenic glucose absorption by measuring jejunal transmural potential differences (PDs) was identical with that previously described (Read *et al.*, 1974). In brief, an intestinal tube was swallowed and positioned with its tip approximately 5 cm beyond the duodeno-jejunal flexure. An intramuscular injection of 30 mg propantheline bromide (Pro-banthine) was then administered. This reduced the normal fluctuations in the record, which varied in amplitude up to 10 mV, to levels (less than 1 mV) sufficiently stable for the measurement of small changes in PD. The solutions shown in Table 1 were infused at a constant rate of 5 ml per minute and the resultant changes in PD between the flowing intraluminal electrode and subcutaneous reference electrode were recorded. A typical record obtained from a patient with untreated coeliac disease is shown in Fig. 1. The infusion of solution 1, containing mannitol but no glucose, provided a baseline PD with the lumen a few millivolts positive to the subcutaneous reference site. Subsequent infusion of a series of solutions containing increasing concentrations of glucose (Table 1) caused the lumen to become progressively

| Solution | Sodium chloride (mM) | Glucose (mM) | Mannitol (mM) |
|----------|----------------------|--------------|---------------|
| 1 | 104 | 0 | 100 |
| 2 | 104 | 10 | 90 |
| 3 | 104 | 20 | 80 |
| 4 | 104 | 50 | 50 |
| 5 | 104 | 100 | 0 |

**Table 1**  *Solutions infused into jejunal lumen*

more negative. After a change in solution the PD usually achieved a new plateau within five minutes. The value of the PD for any infusion was obtained by drawing a straight line through the middle of the record after a plateau had been maintained for 10 minutes. The glucose transfer PD was taken as the difference between the PD level obtained during the infusion of any glucose solution and the baseline obtained during the infusion of solution 1. The total infusion time required for this method never exceeded 90 minutes. The incorporation of a biopsy capsule (Crosby and Kugler, 1957) enabled a specimen of jejunal mucosa to be obtained immediately after completion of the electrical recordings.

Because of the possible inaccuracies of the double reciprocal plot (Lineweaver and Burk, 1934) estimates of 'Apparent Km' (the half saturation constant of the electrogenic mechanism) and PD max (the theoretical maximum transfer PD) were obtained from the electrical data by the additional methods of the direct linear plot (Eisenthal and Cornish-Bowden, 1974) and an unweighted iterative method for a Wang 700 desk computer (Wang programme No. 3504). On the assumption that the parameters are normally distributed, differences between 'Apparent Km' and PD max were evaluated by the appropriate paired or unpaired $t$ test.

**Results**

The glucose transfer PDs in the untreated coeliac and control groups are compared in Fig. 2. The transfer PD generated by 10 mM glucose was very much higher in patients with untreated coeliac disease ($p < 0.0001$). At 20 mM ($p < 0.005$) and 50 mM ($p < 0.05$) the differences in PD become less significant until at 100 mM the transfer PDs were virtually identical. Treatment of the coeliac patients for less than three months caused a reduction in the transfer
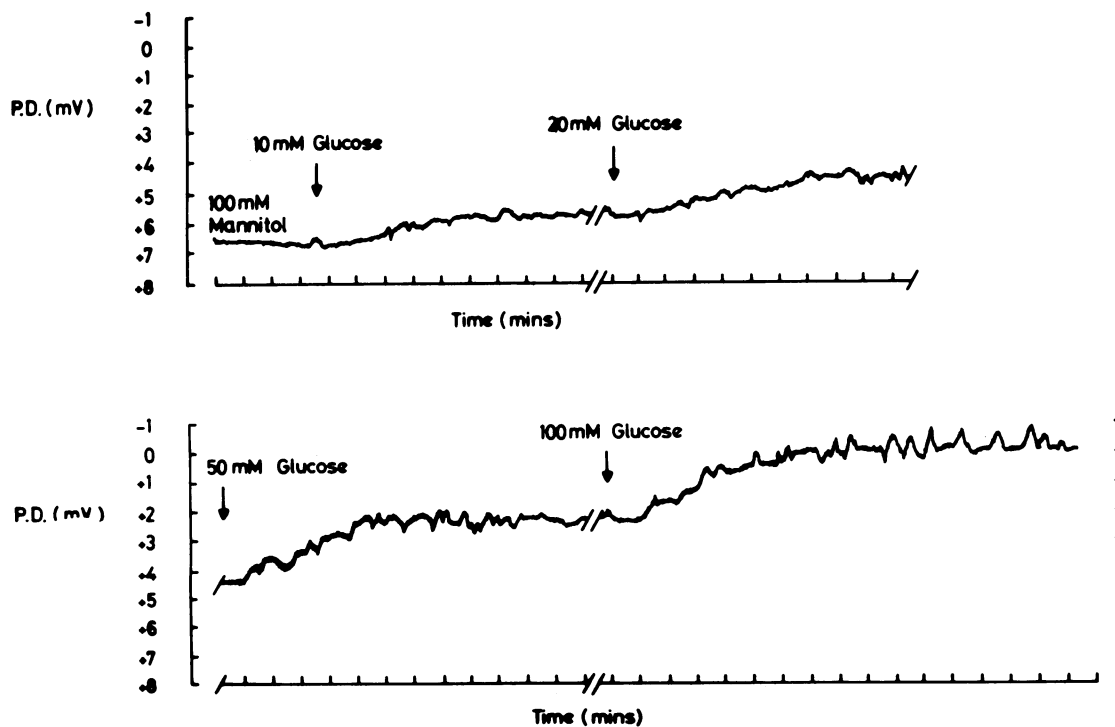


**Fig. 1**  *Tracing of a typical record, taken from a patient with untreated coeliac disease. The full length of the PD plateau for each infusion is not shown in this figure.*
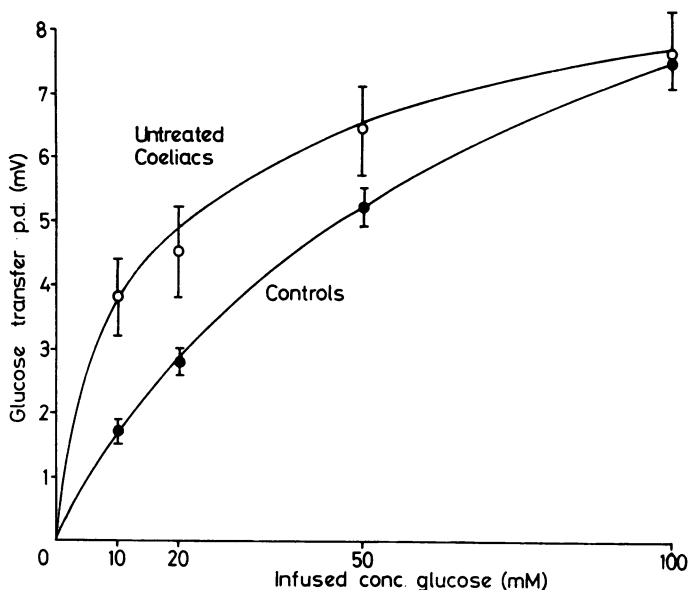
Fig. 2 *Plot of glucose transfer PD against infused glucose concentration in nine patients with untreated coeliac disease and 20 normal control subjects. The results are expressed as means ± SEM.*

PDs at 10 mM and 20 mM glucose but an increase at 100 mM (Fig. 3). After six months' treatment the transfer PD at each glucose concentration was not significantly different from the corresponding value in the control subjects.

The operational kinetic parameters estimated from these data by the three different methods are listed in Table 2. Despite the fact that the values of 'Apparent Km' and PD max obtained from the direct linear plot are medians while those from the other methods are means, there was no significant difference between the results from the three methods. Whatever the method of estimation, the 'Apparent Km' from the untreated coeliac group
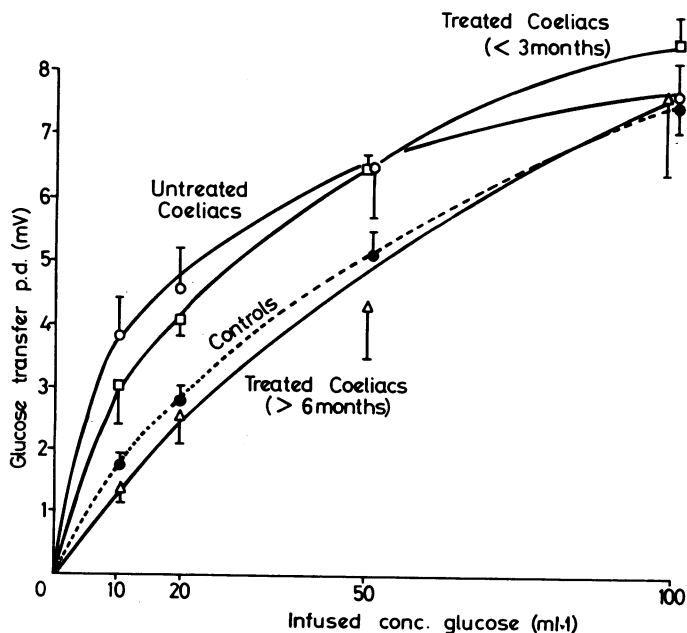


Fig. 3 *Plot of glucose transfer PD against infused glucose concentration in nine patients with untreated coeliac disease, five treated by gluten withdrawal for less than three months and five treated for more than six months. For comparison the results from 20 normal control subjects are included. To avoid confusion some of the points are slightly offset along the abscissa. The results are expressed as means ± SEM.*

were strikingly lower than those from the controls (p < 0·005). Similarly, the values for the PD max were also very much lower in untreated coeliac disease (p < 0·01). Treatment of the coeliac patients for less than three months induced a significant rise in both the 'Apparent Km' (p <0·002) and the PD max (p < 0·02) compared with the untreated group. After six months' treatment there was an additional significant increase in 'Apparent Km' (p < 0·005) and an insignificant increase in PD max (p > 0·05) to levels above those obtained in the control group. The changes in the kinetic parameters upon treatment of the coeliac patients, who were tested on more than one occasion, were accompanied by corresponding improvements in clinical state and jejunal morphology (Table 3).

## Discussion

This study has shown clearly that the operational

kinetic parameters of electrogenic glucose absorption, no matter how calculated, are greatly affected by coeliac disease. The 'Apparent Km' is dramatically decreased while the PD max is also significantly reduced. On treatment with a gluten-free diet the 'Apparent Km' and PD max return to normal levels concomitant with restoration of normal structure (Table 3). In the group treated with a gluten-free diet for more than six months the 'Apparent Km' was significantly greater than that observed in healthy controls but the PD max was not significantly higher. Whether this enhanced 'Apparent Km' has a functional significance is as yet unknown. The actual dose response curve for electrogenic glucose absorption (Fig. 3) for these treated patients is not significantly different from that for normal subjects and, in general, the higher the values for 'Apparent Km' the less meaningful becomes the functional significance of differences of this parameter.

The method of characterising intestinal absorption

| Operational parameter | Method | Groups | | | |
|---|---|---|---|---|---|
| | | Control | Coeliac disease | | |
| | | | Untreated | Treated 3/12 | Treated 6/12 |
| 'Apparent Km' | 1 | 74 ± 5 | 17 ± 2 | 35 ± 6 | 108 ± 13 |
| (mM) | 2 | 69 ± 5 | 20 ± 3 | 29 ± 3 | 106 ± 15 |
| | 3 | 80 ± 8 | 19 ± 3 | 32 ± 8 | 124 ± 22 |
| PD max (mV) | 1 | 12·8 ± 0·9 | 8·6 ± 0·7 | 11·4 ± 1·2 | 15·6 ± 2·7 |
| | 2 | 12·9 ± 1·0 | 8·7 ± 0·7 | 11·0 ± 0·8 | 15·4 ± 2·4 |
| | 3 | 13·6 ± 1·0 | 8·8 ± 0·7 | 10·8 ± 1·1 | 16·6 ± 2·6 |
| n | | 20 | 9 | 5 | 5 |

**Table 2** *Operational kinetic parameters of 'Apparent Km' and PD max for active electrogenic glucose absorption in normal subjects and patients with coeliac disease*

These parameters are calculated from the same data by three different methods. (1) the double reciprocal plot of Lineweaver and Burk (1934); (2) the direct linear plot of Eisenthal and Cornish-Bowden (1974); (3) an unweighted iterative method for a Wang desk computer. The results are expressed as mean ± SEM.

| Patient, sex, age (yr) | Period of gluten withdrawal | Weight (kg) | Haemoglobin (g/dl) | Biopsy appearance | 'Apparent Km' (mM) | PD max (mV) |
|---|---|---|---|---|---|---|
| L.B. | Untreated | 51 | 13·4 | Convoluted | 16 | 8 |
| F 23 | 4 weeks | 52 | 14·5 | Convoluted | 24 | 10 |
| H.F. | Untreated | 64 | 9·8 | Flat | 18 | 8 |
| M 44 | 6 weeks | 67 | 13·0 | — | 48 | 11 |
| | 1 year | 67 | 14·0 | Leaves | 125 | 10 |
| I.T. | Untreated | 56 | 11·8 | Flat | 26 | 10 |
| F 47 | 11 months | 60 | 14·0 | Leaves | 86 | 13 |
| G.M. | Untreated | 58 | 8·1 | Flat | 26 | 9 |
| M 47 | 8 weeks | 62 | 14·4 | Flat | 28 | 10 |
| | 6 months | 75 | 15·0 | Convoluted | 90 | 11 |
| C.W. | 6 weeks | 64 | 11·3 | Flat | 52 | 16 |
| M 59 | 1 year | 70 | 14·7 | Leaves | 91 | 22 |

**Table 3** *Clinical data and operational kinetic parameters of electrogenic glucose absorption determined by method of Lineweaver and Burk, from five patients with coeliac disease before and at varying intervals after initiation of treatment by dietary gluten withdrawal*

The biopsy appearance relates to the gross morphology as seen under the low power ( × 25) of a dissecting microscope. Changes in morphology were always associated with corresponding changes in histological appearance as seen with the light microscope ( × 60).

by the determination of saturation kinetic para-
meters was initially borrowed from studies on
enzyme substrate interactions (Fisher and Parsons,
1953; Smyth, 1971). Its further application to
electrical measurements of transfer activity came
from the *in vitro* studies of Asano (1964), Schultz and
Zalusky (1964), and Lyon and Crane (1966). Debnam
and Levin (1975a, b) extended its application to *in
vivo* studies and showed that the 'Apparent Km' for
the active electrogenic component of glucose
absorption is the same whether calculated from
measurements of hexose transfer PDs or from the
chemically measured loss of glucose from the lumen.
This study suggests that *in vivo* electrical and
chemical methods can be used to characterise the
same process.

A controversial point concerning values of
'Apparent Km' and PD max is whether they can
ever be used as indices of carrier affinity and transfer
capacity respectively. If they can, it would allow
inferences to be made about the effects of coeliac
disease on absorption mechanisms. One possible
way of investigating this appealing prospect is to see
whether the changes in electrical parameters deter-
mined in our study are matched by corresponding
changes in chemical parameters in man. At present
we have found that it is technically impossible to
design a satisfactory protocol to measure accurately
glucose absorption both chemically and electrically
during the same experiment. This is because the
probanthine required to obtain satisfactory stability
for electrical recording makes quantitative recovery
of infusions containing glucose from a site lower down
the intestine extremely variable. Two previous
perfusion studies have, however, measured the dis-
appearance of glucose from the jejunal lumen of
normal and coeliac subjects at several infused con-
centrations (Schedl and Clifton, 1961; Holdsworth
and Dawson, 1965). Both studies showed a decrease
in maximal glucose absorption (Jmax) in the
untreated coeliac groups compared to normal con-
trols. The data of Holdsworth and Dawson (1965),
moreover, shows that the absorption curve in the
untreated coeliac group clearly saturates at a much
lower concentration of glucose than the controls and
thus has a lower 'Apparent Km'. Therefore, there is
a clear association in untreated coeliac disease
between decreases in Jmax and PD max and also
reductions in chemical and electrical 'Apparent
Kms'. While such associations can never be regarded
as proof of a causal relationship, it is more than
tempting to suggest that the changes in the electrical
data are concomitant with the changes in glucose
absorption, measured chemically.

Kinetic parameters, however, whether obtained by
chemical or electrical techniques are affected by a

number of experimental complications. Among
these is the thickness of the unstirred water layer
through which glucose has to diffuse before being
actively transferred by the enterocyte. This has been
shown to exert a profound influence on experimental
values for Km and Jmax *in vitro* (Winne, 1973;
Wilson and Dietschy, 1974; Dugas *et al.*, 1975). By
extrapolation of these data, it is possible that the
reduction in the operational electrogenic parameters
in coeliac disease (Km, PD max) could arise from
attenuation of the unstirred layer.

The decreased PD max observed in untreated
coeliac disease may be due not only to changes in
the ion-linked active transfer mechanisms, but also
to alteration in intestinal mucosal resistance. No
measurements of this resistance have been made in
coeliac disease even *in vitro* and it is unlikely that any
simple technique could be applied to assess it *in vivo*.
However, studies have shown that *in vivo* the jejunal
mucosa in this condition exhibits a greater resistance
to the diffusional movements of several solutes
(Fordtran *et al.*, 1967). While there is no proven
direct correlation between diffusional and electrical
resistances, the data do not support the possibility
that the observed low PD max in coeliac disease may
be due to low mucosal resistance.

Other factors affecting the values of 'Apparent
Km' and PD max are technical, such as the possible
reduction of the effective glucose concentration by
pooling of the serially infused solutions. Because the
electrogenic absorption curve appears to exhibit
saturation kinetics, the PDs obtained at low glucose
concentrations are liable to be lowered more by
pooling than those obtained at the higher concentra-
tions, near saturation level. Thus conditions in which
there is increased pooling alter the shape of the
absorption curve and lead to higher experimental
values for Km and PD max. All the available
evidence indicates that there is greater possibility of
pooling in coeliac disease compared with normal
subjects, in that there is less absorption of solute and
water (Schedl and Clifton, 1961; Holdsworth and
Dawson, 1965), and more secretion (Fordtran *et al.*,
1967; Schmid *et al.*, 1969; Russell *et al.*, 1972) while
the jejunal tone and motility are reduced (Ingelfinger
and Moss, 1943; Ritchie and Salem, 1965). The fact
that the 'Apparent Km' and PD max are actually
lower in untreated coeliac disease strongly suggests
that pooling does not account for the observed
differences between this group and the controls.

In conclusion, despite the uncertainties in inter-
pretation of kinetic parameters for electrogenic
glucose transfer, the changes found in coeliac disease
are qualitatively similar to those found by perfusion
techniques in which glucose absorption was mea-
sured chemically. The total perfusion time for such

chemical kinetic studies is much longer, being a minimum of five hours as compared with 90 minutes for the electrical test. The real meaning of the kinetic parameters obtained by either method is still subject to debate, but data obtained previously in the rat suggests that the electrical method might even give more accurate information about active glucose transport than the chemical method which does not discriminate between active and passive glucose absorption. The narrow single lumen tube used for the electrical method involves no more discomfort than that used for jejunal biopsy, which can indeed be carried out at the end of the test. It thus compares favourably with the four lumen tube used currently for the chemical method. Clearly, a considerable amount of work is required before the electrical test could be advocated for routine clinical usage but at present it provides a useful research tool for the further exploration of absorptive pathophysiology.

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