Changes in intestinal absorption of nutrients and brush border glycoproteins after total parenteral nutrition in rats

S Miura, S Tanaka, M Yoshioka, H Serizawa, H Tashiro, H Shiozaki, H Imaeda, M Tsuchiya

Abstract
The effect of total parenteral nutrition on nutrients absorption and glycoprotein changes of brush border membrane was examined in rat small intestine. In total parenteral nutrition rats, a marked decrease in activity of brush border enzymes was observed mainly in the proximal and middle segments of the intestine. Gastric perfusion of jejunal segment showed that hexose absorption was significantly inhibited, while intestinal absorption of glycine or dipeptide, glycyglycine was not significantly affected by total parenteral nutrition treatment. When brush border membrane glycoprotein profile was examined by [3H]-glucosamine or [3H]-fucose incorporation into jejunal loops, significant changes were observed in the glycoprotein pattern of brush border membrane especially in the high molecular weight range over 120 kDa after total parenteral nutrition treatment, suggesting strong dependency of glycoprotein synthesis on luminal substances. Molecular weight of sucrose isomaltase in brush border membrane detected by specific antibody showed no significant difference, however, in total parenteral nutrition and control rats. Also, molecular weight of specific sodium glucose cotransporter of intestinal brush border membrane detected by selective photoaffinity labelling was not altered in total parenteral nutrition rats. It may be that prolonged absence of oral food intake may produce significant biochemical changes in brush border membrane glycoprotein and absorptive capacity of small intestine, but these changes were not observed in all brush border membrane glycoproteins.

Ingestion of food and the presence of nutrients in the intestine appear to play an essential role in the maintenance of normal small bowel structure and function. It is known, for example, that when exogenous luminal nutrition is completely excluded from the intestine of animals, mucosal hypoplasia develops in the small intestinal mucosa. Total parenteral nutrition, unlike starvation, is able to examine the effect of exclusion of luminal feeding without altering total body energy and nitrogen balance.

Brush border membrane of small intestinal absorptive cells contains various hydrolases and carrier molecules and is known to be the most biologically active site where assimilation of dietary nutrients takes place. Although total parenteral nutrition affects the small bowel morphology and function, there is relative paucity of data concerning how total parenteral nutrition produces changes in biochemical and biophysical properties in the brush border membrane. The aim of this study is therefore to compare the absorptive function of different kinds of nutrients in the rat small intestine maintained with total parenteral nutrition and also to elucidate whether any biochemical changes occur in the glycoproteins and glycoenzymes of brush border membrane of these animals.

Methods
ANIMALS AND METHOD FOR TOTAL PARENTERAL NUTRITION
Male Wistar rats initially weighing 200 g were used for these experiments. The technique used for total parenteral nutrition was a modification of the one described by Steiger, Vars, and Dudrick. Briefly, an indwelling silastic catheter was passed through the jugular vein into the right atrium. The intravenous feeding line was attached to the swivel and to the rat by a harness. Intravenous nutrients were infused throughout the 24 hour period with an infusion pump (Truth type B-II., Tokyo) at 30 ml/day in the beginning and gradually increased to an infusion rate of 50 ml/day by the beginning of the fourth postoperative day. Nutrients solution contains: glucose 206 g/l, amino acids 33 g/l, electrolytes (Na+, K, Cl, Mg, Ca, P), vitamins, trace elements and essential fatty acids. Control rats were sham operated and fitted with harness assemblies similar to those fastened to the backs of parenterally fed rats. Control rats were orally fed with the same amount of nutrient solution per day served ad libitum. The metabolic tolerance to this regimen was monitored by the general appearance of the animal, by blood test and by urine testing to exclude glycosuria, proteinuria, and aciduria.

TISSUE PREPARATION AND ENZYME ASSAY
Fourteen days after initiation of total parenteral nutrition, intestinal absorption and glycoprotein synthesis were determined in parenterally and enterally fed rats. A 10 cm jejunal segment distal to the ligament of Treitz, a 10 cm middle small intestinal segment and a 10 cm ileal segment ending 5 cm from the caecum were removed. The mucosa was scraped from each segment and brush border membrane was prepared by the method of Kessler et al. Aminopeptidase N activity was measured with 2 mM leu-β-naphthylamide as substrate using previously described procedures. Alkaline phosphatase was assayed as described by Fujita et al. Activities...
of intestinal disaccharidas were monitored
according to the method of Dahlvist.16 Protein
was measured by the method of Lowry et al.17

IN VIVO PERFUSION STUDY
In vivo perfusion of galactose, glycine, and
glycylglycine (gly-gly) were performed using a
steady-state perfusion technique.14 Galactose (16
mM or 32 mM), glycine (20 mM or 50 mM), gly-
gly (10 mM) (Sigma, MO, USA) were prepared in
isotonic saline (the osmolarity of all solutions
was adjusted to 350 mosmol/kg by sodium
chloride with osmometer), and the pH was
adjusted to 7.4 and [14C]-polyethylene glycol
(New England Nuclear, Boston, MA, USA) was
added to the solution as a non-absorbable volume
marker. The solution was perfused into a 20 cm
long middle jejunal segment at a rate of 19 ml/h.
After an equilibration period of 30 minutes to
achieve steady state conditions, the perfusate was
collected for three consecutive 10 minute periods.
At the end of each experiment, the perfused intestinal segment was removed and the
mucosa was scraped and weighed. Galactose in
the perfusate was estimated by the method of
Batt and Peters.19 Glycine and gly-gly in the
perfusate were determined by amino acid
analysers as described previously.18 The absorp-
tion rate was expressed as μmol of substrate
absorbed per minute per g mucosa of intestine.

GLYCOPROTEIN PATTERN OF BRUSH BORDER
MEMBRANE BY SODIUM DODECYL SULPHATE,
POLYACRYLAMIDE GEL ELECTROPHORESIS AND
AUTORADIOGRAPHY
Glycoprotein pattern of intestinal brush border
membrane was compared in control and total
parenteral nutrition rats using metabolic label-
ing technique with [3H]-glucosamine and [3H]-
fucose. D-[6-3H]-glucosamine hydrochloride
(specific activity: 20–30 Ci/mmol) and L-[6-3H]-
fucose (specific activity: 10–15 Ci/mmol) were
obtained from New England Nuclear (Boston,
MA, USA). Five hundred microcuries of isotope
was instilled intraluminally into a ligated 20 cm
segment of jejunum by injection with a 26 gauge
needle. The abdominal cavity was closed, and the
animals were allowed to recover. These rats were
killed four hours after injection of the isotope,
and jejunal segments were removed and flushed
thoroughly with ice cold saline. Mucosal scrap-
ings were prepared and homogenised in 40 ml 2
mM Tris-HCl-50 mM mannitol buffer, pH 7.1.
Brush border membranes were subsequently
isolated and subjected to sodium dodecyl
sulphate polyacrylamide gel electrophoresis as
described previously.19 Membranes were first
solubilised by heating in 2%-sodium dodecyl
sulphate with 5%-2-mercaptoethanol for five
minutes at 100°C and then applied to the 7%-
gels. Gels containing [3H]-labelled proteins were
immersed in a autofluor solution for two hours,
dried, and exposed to radiograph film (Kodak
X-omat R Film).

DETERMINATION OF MOLECULAR WEIGHT OF
SUCRASE ISOMALTASE IN BRUSH BORDER
MEMBRANE
Two hundred microcuries of [3S]-methionine
(specific activity: 84 Ci/mmol) obtained from
New England Nuclear (Boston, MA, USA) was
injected into a ligated jejunal segment as de-
scribed above. Four hours after the injection, the
homogenate was made from mucosa of jejunal
segment and brush border membrane was
isolated. Brush border membranes containing
1×106 cpm of trichloroacetic acid-insoluble[3S]-
methionine were solubilised with 1% Triton
X-100 and immunoprecipitation was performed
with rabbit antirat intestinal sucrase isomaltase
(provided from Dr T Goda)20 as described by
Gum et al. The immunoprecipitate was washed
and subjected to sodium dodecyl sulphate poly-
acrylamide gel electrophoresis.

DETECTION OF SPECIFIC GLUCOSE TRANSPORTER
Specific glucose transporter of intestinal brush
border was detected by sodium dodecyl sulphate
gel of fluorescein isothiocyanate labelled vesicles
according to the method of Peerce and Wright.21
Briefly, vesicles (250 μg) from control and total
parenteral nutrition treated rat jejunum were
treated with 2 mM phenyl isothiocyanate (Sigma)
in the presence of 10 mM glucose and 100 mM
NaCl in 50 mM Tris-HCl pH 9.2 and 2 mM
EDTA for 30 minutes at 22°C. Vesicles were
then treated with 50 μM fluorescein isothio-
cyanate (Sigma) in the absence of 10 mM glucose
in the dark at 22°C for 15 minutes. Vesicles were
suspended in 10 mM Tris HCl pH 6.8, 1%,
sodium dodecyl sulphate and β-mercaptoethanol
for 15 minutes at room temperature and centri-
fuged. Electrophoresis was performed on 7% slabs gels according to the method of Laemmli.22

| TABLE 1 | Mucosal weight, brush border membrane protein and brush border membrane enzyme activities of small intestine in rats with total parenteral nutrition |
|-----------------------|-------------------------------------------------|----------------------|---------------------|
| Proximal segment       | Middle segment                                  | Distal segment |
| Intestinal mucosal weight (mg/cm intestinal length) | 63±1 (6.2) | 23±3 (3.3%) | 52±5 (6.0) | 20±2 (3.1%) | 44±2 (3.3) |
| Brush border membrane protein (mg/g mucosa) | 1±0.2 (0.22) | 1±0.7 (0.18), ns | 1±0.5 (0.28) | 1±0.7 (0.34), ns | 1±0.7 (0.30) |
| Enzyme activities (μmol/min/mg protein) | 4±1 (7.6) | 18±4 (5.2%) | 12±6 (3.0) | 7±8 (3.5) | 1±0 (1.5) |
| Alkaline phosphatase | 1±0 (0.29) | 1.5±0.2 (0.30) | 1.5±0.2 (0.10) | 1.5±0 (0.20) | 0.8±0.2 (0.18) |
| Sucrase-isomaltase | 12±3 (1.9) | 8±4 (1.7%) | 11±6 (1.7) | 6±8 (1.4%) | 6±1 (0.20) |
| Amino peptidase N | 5±0 (0.26) | 0±0 (0.28) | 0±0 (0.23) | 0±0 (0.20) | 0.7±0.2 (0.26) |

Mean (SD) of seven experiments *p<0.05, **p<0.01, ***p<0.001, as compared with control values. ns not significant; TPN= total parenteral nutrition.
After electrophoresis, fluorescein isothiocyanate in the slices was determined by spectrofluorometer (JASCO, FP-550, Tokyo).

STATISTICAL ANALYSIS
Data were analysed for significance of difference using Student's t test.

Results
ANIMAL CHARACTERISTICS
During total parenteral nutrition treatment, there was no significant difference in the increase of body weight between control and total parenteral nutrition treated rats and after 14 days, the weight gain being 49 (5) g in controls and 45 (6) g in total parenteral nutrition treated rats. There were also no significant differences in serum total protein, albumin, cholesterol, blood sugar, urea nitrogen, and haemoglobin concentrations between two groups, indicating that systemic metabolic changes did not occur throughout total parenteral nutrition period as judged by these parameters.

BRUSH BORDER MEMBRANE PROTEIN AND ENZYME ACTIVITIES
As shown in Table I, there was marked decrease in intestinal mucosal weight throughout the small intestine in total parenteral nutrition rats. When brush border membrane was purified and compared as mg brush border membrane per gram intestinal mucosal protein basis, however, there was no significant difference between two groups in any segment of the intestine, revealing that there was no selective loss of brush border membrane protein compared with other mucosal proteins after total parenteral nutrition treatment. Intestinal brush border enzyme activities are also shown in Table I. In control rats, all enzyme activities of brush border membranes isolated from different regions showed considerable difference along the length of the small intestine. The proximal segment contained three, four times higher activity of alkaline phosphatase than the middle segment and there were very low specific activities in the distal segment. In the case of disaccharidases and aminopeptidase N, these enzymes had a maximum peak in the mid intestine, decreasing to approximately half maximum in the distal end. Total parenteral nutrition treatment appeared to decrease specific activities of all these enzymes mainly in the proximal and middle segment of the intestine. Distal segment was not significantly affected by total parenteral nutrition treatment, resulting in the attenuation of proximal distal gradient of enzyme activities in these brush border enzymes.

INTESTINAL ABSORPTION STUDIES
Table II compared the absorptive function of galactose, glycine, and glycyglycine in vivo by using steady state perfusion technique in control and total parenteral nutrition rat jejunum. When galactose absorption from jejunal loops was compared, significant inhibition of galactose uptake from the mucosa was observed in total parenteral nutrition rats compared with controls in both 16 and 32 millimolar concentrations.

On the other hand, neutral amino acid, glycine and dipeptide, glycyglycine are supposed to be transported by different carrier systems from that of glucose-galactose. When glycine was perfused into the jejunal loops, there was no significant inhibition of glycine transport from intestine after total parenteral nutrition treatment. Our perfusion study also revealed that there was no difference in glycyglycine absorption between total parenteral nutrition and control rat small intestines as shown in Table II.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>In vivo absorption of galactose, glycine and glycyglycine from jejunal perfuse loops in rats with total parenteral nutrition</th>
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<tr>
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<td>Control (μmol/min/g mucosa)</td>
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<tr>
<td>Galactose</td>
<td>16 mM</td>
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<tr>
<td>Glycine</td>
<td>20 mM</td>
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<td>Glycylglycine</td>
<td>10 mM</td>
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Mean (SD) of six experiments *p<0·01 as compared with control values. ns = not significant; TPN = total parenteral nutrition.

Figure 1: Effect of total parenteral nutrition (TPN) on the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profiles of brush border membrane proteins and glycoproteins labelled in vivo with [3H]glucosamine. Left: protein bands stained with Coomassie blue; right: radioautography of brush border membrane glycoproteins labelled with [3H]glucosamine. In all cases 50 μg protein was applied to each lane. Arrowheads indicate increased or decreased staining of protein and [3H]glucosamine labelled glycoprotein bands. Experimental details are described in the text.
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Changes

1. Effect of total parenteral nutrition on the sodium dodecyl sulphate polyacrylamide gel electrophoresis profiles of brush border membrane proteins and glycoproteins labelled in vivo with [3H]-fucose. Left: protein bands stained with Coomassie blue; right: radioautography of brush border membrane glycoproteins labelled with [3H]-fucose. In all cases 50 mg protein was applied to each lane. Arrowheads indicate increased or decreased staining of protein and [3H]-fucose labelled glycoprotein bands. Experimental details are described in the text.

GLYCOPROTEIN PATTERN OF BRUSH BORDER MEMBRANE

It is thought that brush border membrane enzymes and a variety of membrane transporters are glycoproteins. To determine whether there are any biochemical changes in the brush border membrane glycoproteins, glycoprotein pattern after the incorporation of [3H]-labelled carbohydrate into membrane proteins was examined by the autoradiography of sodium dodecyl sulphate gel electrophoresis. As shown in Figures 1 and 2, a control of protein band treatment total parenteral nutrition treated brush border membrane protein profiles on sodium dodecyl sulphate polyacrylamide gels revealed distinct differences. The control jejunal brush border membranes had prominent bands of 140 and 220 kDa, while the total parenteral nutrition treated jejunum showed their decrease. Incorporation of labelled glucosamine and fucose into the brush border membrane was decreased in total parenteral nutrition rats and their total counts incorporated into glycoproteins were about two-thirds of those of control rats as expressed by counts/mg membrane protein. As shown in Figure 1, glycoproteins labelled by [3H]-glucosamine showed intense labelling in the region from 80–250 kDa in the control rat jejunum. There were significant changes in their incorporation pattern after total parenteral nutrition treatment and especially high molecular weight glycoprotein bands over 120 kDa were mainly suppressed in total parenteral nutrition rats. Namely, glucosamine incorporation into 250, 210, 120 and 85 kDa glycoprotein were remarkably decreased in total parenteral nutrition rats, in contrast with the increase of glycoprotein incorporation into 110 kDa glycoprotein. Fucosylated glycoprotein pattern in control and total parenteral nutrition rats is shown in Figure 2. Similar to the [3H]-glucosamine incorporation, there was selective decrease in several bands, mainly glycoproteins of molecular weight over 120 kDa after total parenteral nutrition treatment. In total parenteral nutrition rats, the fucosylated glycoprotein synthesis of 220, 120, 100 kDa was significantly decreased, whereas 110 kDa glycoprotein was rather increased. From these observations, there is possibility that by the total parenteral nutrition treatment, mainly biosynthesis of high molecular weight glycoproteins are suppressed in the brush border membrane of jejunal mucosa.

MOLECULAR WEIGHT OF SUCRASE ISOMALTASE

Sucrase isomaltase isolated from jejunal total parenteral nutrition treated rat contained 120 kDa of sucrose subunit and 140 kDa of isomaltase subunit as judged by sodium dodecyl sulphate polyacrylamide gel electrophoresis in denatured condition. These molecular weights of sucrase and isomaltase were identical to those of control rat (Fig 3).

MOLECULAR WEIGHT OF SPECIFIC GLUCOSE TRANSPORTER

Molecular weight of specific sodium glucose cotransporter of intestinal brush border membrane was analysed by fluorescein isothiocyanate using sodium dodecyl sulphate polyacrylamide gel electrophoresis. The molecular weight of this transporter was 80 kDa, and this molecular weight was not altered after total parenteral nutrition treatment, suggesting that so far as this glucose transporter is concerned, no significant changes in molecular form of transporter had occurred with total parenteral nutrition treatment in spite of the decreased glucose absorption.

Discussion

The brush border membrane of intestinal mucosal cells contains various kinds of glycoconjugates and their carbohydrate moieties have been shown to be involved in many important biological functions. A variety of membrane associated hydrolytic enzymes and transporter proteins play an especially important role in the absorptive process and active transport of dietary nutrients. These enzymes are known to be glycosylated to varying degrees and their process of biosynthesis and expression at the cell surface has recently been investigated. Their regulation has still been poorly defined, however. This study was designed to elucidate changes of brush border function and glycoprotein composition in response to total parenteral nutrition.
control and total parenteral nutrition treated animals. This result suggests the possibility that prolonged absence of dietary stimuli may not alter the major structure of glycoenzyme molecule in brush border membrane, but could affect their rate of biosynthesis.

In our study, the effect of total parenteral nutrition varies depending on the region of the small intestine. The extent of the decrease in brush border membrane enzyme activities was much greater in upper and middle segments of intestine compared with lower segment. It has recently been postulated that glycoproteins from brush border membranes of the proximal and distal intestine are qualitatively different and that the glycoproteins from distal intestine may have more completed oligosaccharide side chains.\textsuperscript{11} We can suppose that the regulatory mechanisms of brush border membrane glycoprotein is different between jejunum and ileum and that those of ileum are more independent from the luminal substances.

Crane correctly inferred earlier that for hexose transport the needed metabolic energy is delivered to the active transport carrier for glucose and galactose by coupling them to the flux of Na\textsuperscript+ moving across the brush border membrane in response to a lumen-cell Na\textsuperscript+ gradient.\textsuperscript{32} In the present study, we have shown that the disappearance rate of two different concentrations of galactose from intestinal segment was significantly inhibited by total parenteral nutrition treatment. Although glucose/galactose transportation was significantly altered by total parenteral nutrition treatment, selective photo-affinity labelling for transporter properties showed that there were no significant changes in their molecular weight. Our results were in agreement with the observation of Kotler et al.\textsuperscript{31} who indicated that luminal nutrients maintain carrier mediated glucose transport after direct contact with the mucosa and that nutrient effect appears to involve an increased number of glucose carriers. On the other hand, glycine and glycyglycine absorption was not altered by the total parenteral nutrition treatment. As single or two substrate concentrations were used in these perfusion studies, it is difficult to interpret the characteristics of absorption such as carrier number or affinity. It is now known that there are multiple transporters for amino acids with overlapping specificity. At least four different Na\textsuperscript+ dependent transport systems for neutral amino acids have been described for brush border membrane of the small intestine.\textsuperscript{14} Moreover, the transport systems for peptides differ from those for amino acids, but the number of these systems has not been clarified.\textsuperscript{14} Hoshi et al showed the relative importance of intact dipeptide transport in protein assimilation and suggested that glycyl-L-leucine uptake by guinea pig small intestine is actively transported through H\textsuperscript+ ion dependent carrier system.\textsuperscript{14} The exact reason why total parenteral nutrition did not affect glycine and glycyglycine absorption was not known. It may be justified to say, however, that the multiplicity of systems seems to work as a protective mechanism to ensure the adequate uptake of peptides. There might be complex interrelationships existing between the presence of luminal nutrients

In total parenteral nutrition treatment, significant changes occurred in the glycoprotein pattern of brush border membrane, especially those of higher molecular weight over 120 kDa in our study of radiolabelled sugar incorporation. Decrease in these glycoproteins suggests the possibility that glycoproteins in brush border membrane were continuously stimulated by the luminal substances which have direct contact with mucosa. As four hours after the administration of radiolabelled sugar, these glycoproteins are supposed to be just processed to the brush border membrane at that time,\textsuperscript{14} these changes by total parenteral nutrition may reflect the alteration of their de novo biosynthesis rather than the changes in their degradation process. This considerable heterogeneity in the changes in biosynthesis of brush border membrane glycoproteins may correlate their different response against dietary stimuli. When we examined sucrase isomaltase as a brush border membrane enzyme, however, there was no difference in the molecular weight of sucrase isomaltase between

Figure 3: Immunopurified sucrase isomaltase (SI) from intestine of total parenteral nutrition (TPN) and control rat. Sucrase isomaltase molecules were determined by rabbit antirat intestinal sucrase isomaltase from \textsuperscript{35}S]-methionine-labelled brush border membrane as described in the text. Molecular weight of S and I was approximately 120 kDa and 140 kDa respectively. No difference in molecular weight of sucrase isomaltase between total parenteral nutrition treated and control rat was observed.
and the regulation of nutrient absorption. As we have shown that there are indeed considerable heterogeneities of total parenteral nutrition effect among different glycoenzymes and transporter proteins, there should be many different kinds of regulatory signals such as transported substrate itself and neurohumoral substances participating in the biosynthesis of glycoproteins in the brush border membrane of rat small intestine. It remains to be elucidated which of the regulatory signals is important in the physiological condition.

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